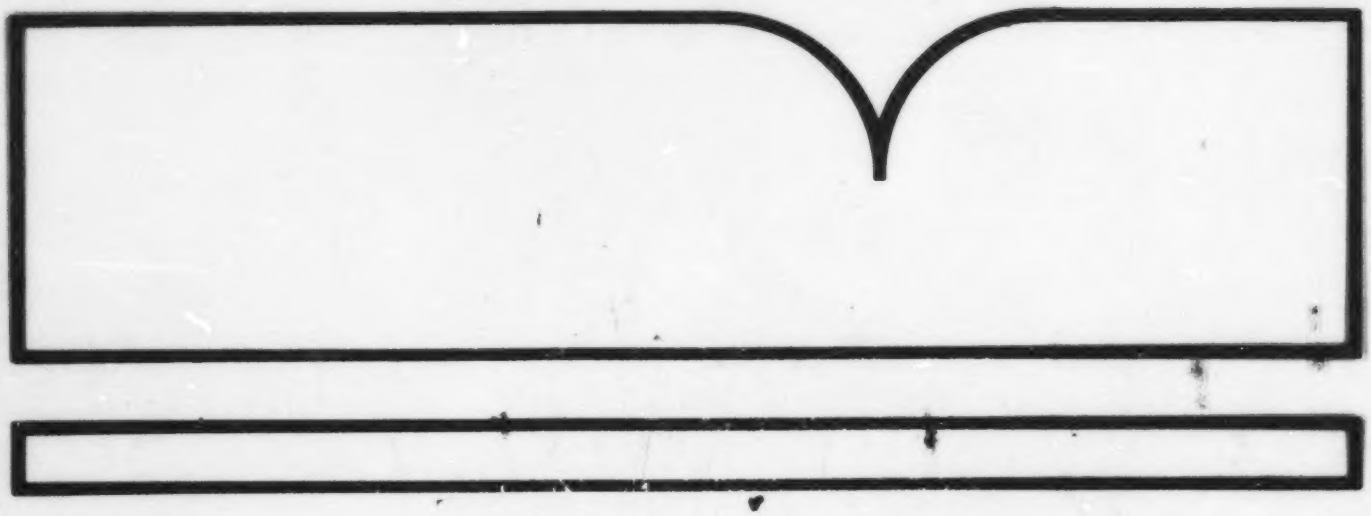
PB83-149120

Effects of Kelthane and Pydrin on Early Life Stages of Fathead Minnows (Pimephales promelas) and Amphipods (Hyalella azteca)

(U.S.) Environmental Research Lab.-Duluth, MN

1982



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO EPA-600/D-82-361	2. Published Paper	3. RE PB83-149120
4. TITLE AND SUBTITLE Effects of Kelthane and Pydrin on Early Life Stages of Fathead Minnows (Pimephales promelas) and Amphipods (Hyalella azteca)		5. REPORT DATE 1982 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Spehar, R. L., D. K. Tanner, and J. H. Gibson		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, Minnesota 55804		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as above		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA/600/03

15. SUPPLEMENTARY NOTES
IN: Aquatic Toxicology and Hazard Assessment: Fifth Conference, ASTM STP 766, J.G. Pearson, R.B. Foster, and W.E. Bishop, Eds., Am. Soc. Test. Mater., pp. 234-244 (1982).

16. ABSTRACT

Embryonic, larval and early juvenile stages of fathead minnows (Pimephales promelas) and juvenile amphipods (Hyalella azteca) were exposed to Kelthane, and fathead minnows were exposed to Pydrin in a small self-contained continuous-flow diluter system for approximately 30 days. Survival of the newly hatched larvae was found to be the most sensitive measure of the effect on fathead minnows of both pesticides. Pydrin was approximately 90 times more toxic to fathead minnows than Kelthane. The lowest effect concentrations of Kelthane and Pydrin were 13 times lower than the 96-h median lethal concentration (LC50) values for fathead minnows exposed in Lake Superior water. The upper and lower chronic limits for Kelthane were 39 and 19 µg/litre for fathead minnows and 33 and 19 µg/litre for amphipods. The chronic limits for Pydrin and fathead minnows were 0.43 and 0.33 µg/litre. The mean bioconcentration factors (BCFs) for Kelthane were 3,700 with fathead minnows and 10,000 with amphipods. The mean BCF for Pydrin with fathead minnows was 3,200. Residue concentrations and BCFs for both pesticides increased with increased water concentrations.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED 20. SECURITY CLASS (This page) UNCLASSIFIED	21. NO. OF PAGES 12 22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/D-82-361
PUBLISHED PAPER

R. L. Spehar,[1] *D. K. Tanner,*[1] *and J. H. Gibson*[2]

Effects of Kelthane and Pydrin on Early Life Stages of Fathead Minnows (*Pimephales promelas*) and Amphipods (*Hyalella azteca*)

REFERENCE: Spehar, R. L., Tanner, D. K., and Gibson, J. H., "**Effects of Kelthane and Pydrin on Early Life Stages of Fathead Minnows (*Pimephales promelas*) and Amphipods (*Hyalella azteca*),**" *Aquatic Toxicology and Hazard Assessment: Fifth Conference, ASTM STP 766*, J. G. Pearson, R. B. Foster, and W. E. Bishop, Eds., American Society for Testing and Materials, 1982, pp. 234–244.

ABSTRACT: Embryonic, Larval, and early juvenile stages of fathead minnows (*Pimephales promelas*) and juvenile amphipods (*Hyalella azteca*) were exposed to Kelthane, and fathead minnows were exposed to Pydrin in a small self-contained continuous-flow diluter system for approximately 30 days. Survival of the newly hatched larvae was found to be the most sensitive measure of the effect on fathead minnows of both pesticides. Pydrin was approximately 90 times more toxic to fathead minnows than Kelthane. The lowest effect concentrations of Kelthane and Pydrin were 13 times lower than the 96-h median lethal concentration (LC_{50}) values for fathead minnows exposed in Lake Superior water. The upper and lower chronic limits for Kelthane were 39 and 19 μg/litre for fathead minnows and 33 and 19 μg/litre for amphipods. The chronic limits for Pydrin and fathead minnows were 0.43 and 0.33 μg/litre. The mean bioconcentration factors (BCFs) for Kelthane were 3 700 with fathead minnows and 10 000 with amphipods. The mean BCF for Pydrin with fathead minnows was 3 200. Residue concentrations and BCFs for both pesticides increased with increased water concentrations.

KEY WORDS: Kelthane, dicofol, Pydrin, fenvalerate, pesticides, synthetic pyrethroid, acute toxicity, chronic toxicity, bioaccumulation, early life stage, fresh water, fish, aquatic invertebrates, fathead minnows, amphipods, aquatic toxicology, hazard assessment

The increased manufacture and use of pesticide chemicals may pose a direct threat to water quality by contaminating aquatic systems. Water pollution due to pesticides can occur from industrial discharge, improper disposal, and runoff rain from agricultural and forest applications [*1,2*].[3]

[1] Research aquatic biologist and physical science technician, respectively, U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn. 55804.
[2] Chemist, Department of Chemistry, University of Minnesota, Minneapolis, Minn. 55455.
[3] The italic numbers in brackets refer to the list of references appended to this paper.

Although pesticides have been developed to control unwanted animal and plant life, nontarget organisms, particularly fish and invertebrates important in the aquatic food chain, can be adversely affected by some formulated compounds [*3–8*]. Little or no information is available in the literature on the long-term effects of several pesticides, now being used in various applications, on these aquatic organisms.

The objective of the present study was to predict the long-term no-effect concentration of Kelthane, an acaricide used on a number of vegetable, fruit, and grain crops, and Pydrin, a synthetic pyrethroid used on cotton, vegetable, and fruit crops as well as for mosquito control, in tests with early life stages of the fathead minnow (*Pimephales promelas*). A review of the literature has indicated that tests with embryonic–larval and early juvenile stages are the most or among the most sensitive to numerous chemicals [*9*]. These tests are quick, cost-effective, and have shown a good correlation with results from long-term life cycle tests.

Fathead minnows were selected for study because standard methods for this species have been developed for early life stage tests and because the acute toxicity of both pesticides to this species is known [*10*]. Juvenile amphipods (*Hyalella azteca*) were exposed to Kelthane as a basis for testing invertebrates in a system designed for testing early life stages of fish and because this class of invertebrates had been shown to be sensitive to a variety of pesticides [*3,4*].

Materials and Methods

Water Characteristics

Lake Superior water was filtered through sand, sterilized with ultraviolet light, and heated to 25 ± 2°C for tests with fathead minnows and to 15 ± 2°C for tests with amphipods. Chemical characteristics of the test water were determined weekly [*11*]. Ranges for these measurements [in milligrams per litre as Calcium Carbonate ($CaCO_3$)] were as follows: hardness, 43 to 47; alkalinity, 35 to 45; and acidity, 1.5 to 4.8. The pH ranged from 7.1 to 7.8. Dissolved oxygen concentrations ranged from 6.1 to 8.2 mg/litre in fathead minnow tests and from 8.6 to 9.8 mg/litre in tests with amphipods.

Exposure System

The tests were conducted with a continuous-flow mini-diluter exposure system [*12*] which delivered five pesticide concentrations and a control to four replicate exposure chambers. Duplicate exposures were used with amphipods because of the small number of animals available. Glass test chambers measured 7 cm wide by 19 cm long by 9 cm high, with a water depth of 4.5 cm. The flow rate to each chamber was 12.5 ± 1 ml per minute, providing a 95 percent replacement of the test water every 2 h [*13*]. Sylvania

Cool White fluorescent bulbs provided a light intensity of 2 to 6 lx at the water surface during a 16-h light photoperiod.

Toxicant Solution

Technical-grade Kelthane (common name dicofol, 87 percent active ingredient) (4,4′-dichloro-α-trichloro-methylbenzhydrol) and Pydrin (common name fenvalerate, 97 percent active ingredient) [cyano(3-phenoxyphenyl) methyl 4-chloro-α-(1-methylethyl)benzeneacetate] were supplied by the Rohm and Haas Co., Philadelphia, Pa., and the Shell Chemical Co., Agricultural Chemicals, Houston, Tex., respectively.

Saturated solutions of Kelthane and Pydrin were used as the toxicant source to avoid the use of solvent chemicals. The saturator system for Kelthane consisted of a stainless steel screen column coated with the pesticide which was contained in a pressurized stainless steel canister filled with approximately 19 litres of Lake Superior water [*14*]. A saturated solution (1.6 to 1.8 mg/litre Kelthane) was reached in approximately 5 days after constant stirring. A saturator system modified from that described by Veith and Comstock [*15*] for Pydrin consisted of a glass column packed with sand (containing ~2 percent Pydrin) and a reservoir filled with 11 litres of water. A saturated solution (16 μg/litre Pydrin) was obtained in about 5 days after the solution was recirculated through the column.

Water samples for Kelthane analyses were collected twice a week and extracted with hexane before gas chromatography (GC) analysis occurred. The samples were analyzed on a Hewlett–Packard 5713 A gas chromatograph with an autosampler and a nickel-63 electron capture cell. The column was a 1.8 m by 2-mm internal diameter (ID) glass coil filled with 4 percent SE30 and 6 percent OV-210 on 80/100 mesh Gas Chrom Q. The carrier gas was argon containing 5 percent methane. Analyses were run at a column temperature of 195°C. The limit of detection for this procedure was ~1 μg/litre, and the mean percentage recovery and standard deviation for 19 spiked samples were 91 ± 16. The column used for Pydrin water analysis was a 1 m by 2-mm (ID) glass coil filled with 3 percent OV-7 on 80/100 mesh W-HP Chromosorb. The carrier gas was 5 percent methyl(CH_3)–argon utilizing a temperature program of 220°C, 4°C/min to 250°C. The limit of detection for this method was ~0.02 μg/litre, and the percentage recovery for one spiked sample was ~105. Measured water concentrations (mean ± standard deviation) for Kelthane and Pydrin are shown in Figs. 1 to 3.

Biological Procedures

Tests with fathead minnows were begun by transferring 25 to 30 embryos (less than 1-day-old) from the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory–Duluth culture unit into 120-ml glass jars with 40-mesh nytex screen bottoms and placing them in the ex-

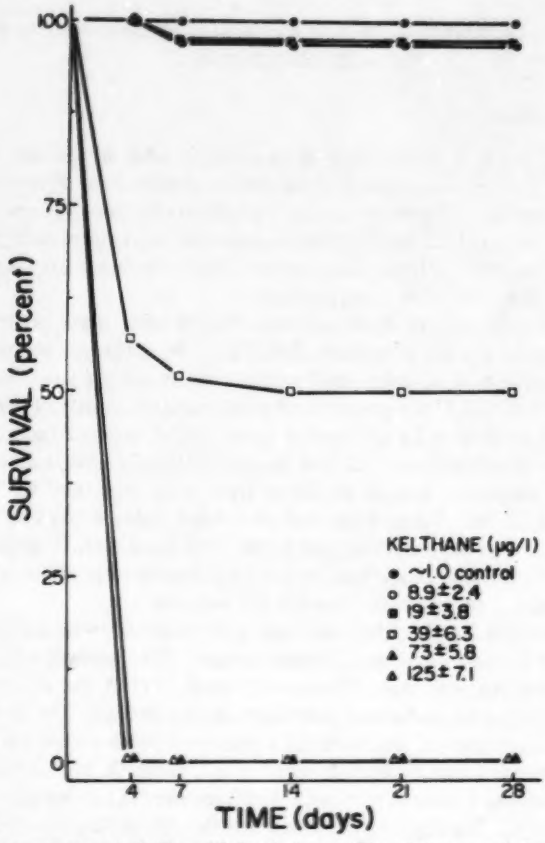


FIG. 1—*Percentage survival of larval fathead minnows* (Pimephales promelas) *after 28 days of exposure to Kelthane. The larvae were previously exposed as embryos.*

posure chambers for calculating percentage hatchability. After all the embryos had hatched (~4 to 5 days), 15 larvae were distributed to randomized, replicate chambers. For the remainder of the test (28 days) all the larvae were fed 3 to 5 ml of brine shrimp nauplii three times a day. The dead larvae were counted daily.

A 28-day test with amphipods was begun by distributing 15 juveniles (1 to 3 mg) to each duplicate chamber. The amphipods were obtained from the Eau Clair River near Gordon, Wisc., and were allowed to acclimate to the test temperature for approximately 2 weeks. They were fed aspen and birch leaves previously soaked for 1 month in Lake Superior water. The soaked leaves were cut into circles (2 cm in diameter) and were fed *ad libitum*.

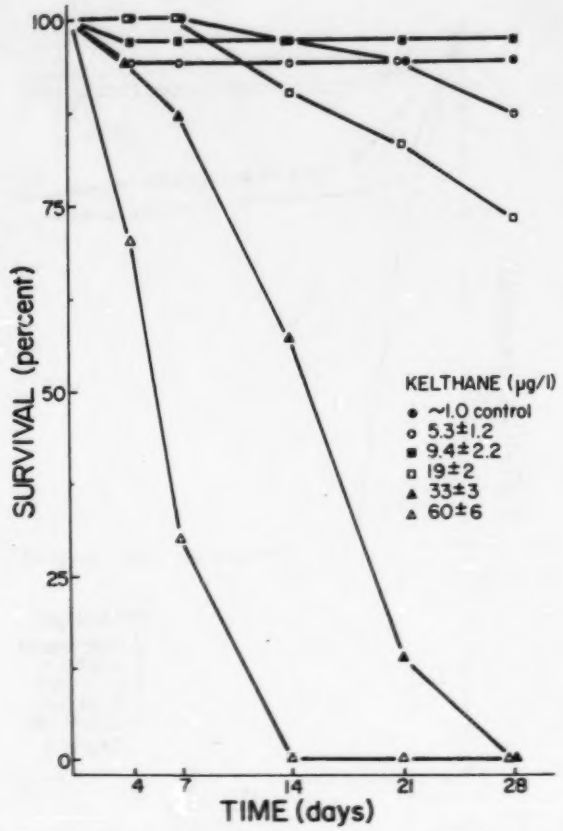


FIG. 2—*Percentage survival of juvenile amphipods* (Hyalella azteca) *after 28 days of exposure to Kelthane.*

Residue Analysis

The fathead minnows and amphipods that survived the tests were frozen before being analyzed [*16*] for Kelthane and Pydrin. Before they were frozen, the fathead minnows were blotted and individually weighed to the nearest milligram for growth determinations. Gas chromatograph conditions for Kelthane and Pydrin analyses were the same as those used for water samples. The mean percentage recovery and standard deviations for these chemicals for three spiked samples were 104 ± 13 and 95 ± 6, respectively. Bioconcentration factors (BCFs) were calculated by dividing the pesticide concentration in whole body tissue by the average measured pesticide concentration in the exposure water. The limit of detection for this method was 1 and 0.02 µg/g for Kelthane and Pydrin, respectively.

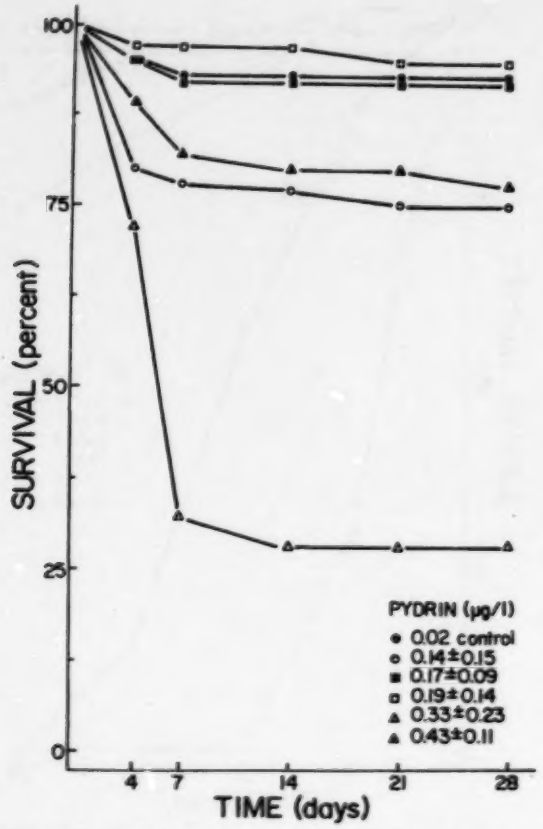


FIG. 3—*Percentage survival of larval fathead minnows* (Pimephales promelas) *after 28 days of exposure to Pydrin. The larvae were previously exposed as embryos.*

Statistical Analysis

Survival and growth determinations were subjected to one-way analysis of variance ($p = 0.05$) and to Dunnett's one-sided comparison of treatment means to the control means ($p = 0.05$) [*17*].

Results

Kelthane

Kelthane did not significantly reduce the total percentage hatchability of fathead minnow embryos but delayed hatching for 1 day at a concentration of 125 µg/litre. Only three fish hatched at this concentration after 4 days, compared with nearly all those exposed to lower concentrations. A signifi-

cant reduction in hatchability was not observed at any concentration by the fifth day of exposure when the larvae were transferred to the larval chambers. The larvae at 125 μg/litre were deformed, and all died within 1 day after hatching. After 4 days, all the larvae exposed to the next lower concentration of 73 μg/litre died, and approximately one half of the larvae exposed to 39 μg/litre died (Fig. 1). Thereafter only a few additional fish died at this concentration. No statistically significant decreases in survival were noted in the fish exposed to Kelthane concentrations averaging 19 μg/litre or less. After the 32-day test, growth (weight) of the larvae was not decreased at any of the concentrations where the fish were still alive.

Survival of amphipods was significantly decreased at 60 μg/litre of Kelthane after 4 days of exposure (Fig. 2), and all the animals died at this concentration after 14 days. After this period, survival was also decreased at 33 μg/litre, and all the amphipods exposed to this concentration were dead by the end of the test. Approximately 30 percent of the amphipods died after exposure to 19 μg/litre, but statistical differences could not be shown. Before death, amphipods usually became periodically immobilized on the bottom of the test chambers and were able to move only their appendages. This behavior alternated with periods of vigorous swimming.

Concentrations of Kelthane in whole fathead minnows are shown in Table 1. Residues in the control animals were not detectable (<1.0 μg/g, wet weight). Mean residue concentrations ranged from 28 to 230 μg/g for fish exposed to 8.9 to 39 μg/litre, respectively. The mean BCF and standard deviation based on residue accumulations at water concentrations causing no adverse effects to fathead minnows were 3 700 ± 800. Kelthane concentrations in whole amphipods are also shown in Table 1. Residue concentrations in control animals were less than detectable. Mean residue concentrations

TABLE 1—*Accumulation of Kelthane and Pydrin in fathead minnows* (P. promelas) *and amphipods* (H. azteca) *after exposure for ~30 days.*

Species	Measured Water Concentration, μg/litre	Measured Tissue Concentration, μg/g, wet weight	BCF
	Kelthane		
Fathead minnow	8.9 ± 2.4[a]	28.0 ± 6.0[a]	3 100
	19.0 ± 3.8	81.0 ± 7.0	4 300
	39.0 ± 6.3[b]	230 ± 94	...
Amphipod	5.3 ± 1.2	33 ± 14	6 200
	9.4 ± 2.2	100 ± 13	11 000
	19.0 ± 2.0	230 ± 49	12 000
	Pydrin		
Fathead minnow	0.14 ± 0.15	0.23 ± 0.03	1 600
	0.17 ± 0.09	0.46 ± 0.04	2 600
	0.19 ± 0.14	0.88 ± 0.18	4 600

[a] Mean ± standard deviation.
[b] Concentration causing significant decreases in survival.

ranged from 33 μg/g for animals exposed to 5.3 μg/litre to 230 μg/g for those exposed to 19 μg/litre. The 28-day mean BCF and standard deviation calculated from this range for amphipods exposed to Kelthane were 10 000 ± 3 000.

Pydrin

Pydrin did not reduce the total percentage hatchability of fathead minnow embryos but did impair the swimming ability of the 1-day-old larvae exposed to 0.43 μg/litre. Percentage survival of larvae at this concentration was decreased from the control after 7 days of exposure (Fig. 3). The next lower concentration of 0.33 μg/litre initially impaired the swimming of some larvae, but they recovered after 7 days. Significant decreases in larval survival were not detected at this or lower concentrations. Growth (weight) was not decreased at any of the concentrations tested.

Concentrations of Pydrin in whole fathead minnows are shown in Table 1. Residue values for control fathead minnows were less than the detection limit of 0.02 μg/g (wet weight). Mean residue concentrations ranged from 0.23 to 0.88 μg/g for fish exposed to 0.14 and 0.19 μg/litre, respectively. Consequently, the mean BCF and standard deviation for fathead minnows were 3 000 ± 1 500.

Discussion

Survival of larvae and early juveniles of the fathead minnows exposed to Kelthane and Pydrin was the most sensitive measure of effect in this study. Concentrations which decreased survival did not impair growth after approximately 30 days of exposure. These results are contrary to those found in exposures with early life stages of fathead minnows and other pesticides, including some encapsulated forms [*18*]. Growth of the larval and early juvenile stages, generally, was the most sensitive indicator of stress in these studies as well as in studies with various organic chemicals [*19,20*]. The differences in the effect end points between these chemicals appears to be related to differences in the mechanisms of their toxic action.

In the present study, the larvae were more sensitive than the embryos to Kelthane and Pydrin. The concentrations that affected survival were nearly three times lower than the concentrations that had no effect on percentage hatchability. Similar resuits were observed for early life stages of fathead minnows exposed to several organic compounds [*19,20*]. Although the reasons for the susceptibility differences in these life stages are not well understood, it appears that toxic substances bind, in various degrees, to the outer egg structures, such as the chorion membrane and yolk, which permits little penetration of the chemical into the embryo [*21*]. Guiney et al [*22*] found that the greatest amount of polychlorinated biphenyls (PCBs) were accumulated in the yolk of eggs of rainbow trout which contain a high fat content

consisting of phospholipids and triglycerides. These authors attributed this to the lipophilic nature of PCBs. Since chlorinated structures such as Kelthane and Pydrin have a high affinity for lipids [*23*], their binding to fat and proteins in the outer egg structures seems to be a logical mechanism of protection which accounts for the tolerance of embryos in this study. The large number of deformed larvae which occurred in the highest Kelthane concentration following hatching suggests, however, that some accumulation does occur in embryos exposed to lethal concentrations during incubation. Holcombe et al [*20*] found that a pentachlorophenol concentration slightly higher than the 96-h median lethal concentration (LC_{50}) value reduced normal embryo hatchability and caused larval deformity.

In this test, Pydrin was approximately 90 times more toxic than Kelthane to fathead minnow larvae. The upper chronic limit (the lowest concentration which caused significant decreases from the control) was 0.43 and 39 μg/litre for these pesticides, respectively. The lower chronic limit (the highest tested concentration which did not cause significant decreases from the control) was 0.33 μg/litre for Pydrin and 19 μg/litre for Kelthane. Comparison of the data showed that these concentrations of both pesticides were approximately 13 times lower than the 96-h LC_{50} values obtained for fathead minnows in acute tests [*10*] in Lake Superior water. The upper and lower chronic limits for Kelthane in amphipods were 33 and 19 μg/litre.

These data indicate that very low concentrations of Kelthane and Pydrin are toxic to early life stages of the tested organisms. Although no information was found on the toxic effect of field application rates of Kelthane to nontarget organisms, a rate of 1.9 kg (4.2 lb) active ingredient/acre [*24*] (used to control mites on fruit, vegetable, and field crops) applied to a water body where the depth ranged from 0.3 to 3.1 m could result in concentrations of 150 to 1 500 μg/litre, assuming a uniform mixing and distribution. These concentrations are much greater than those affecting larval fathead minnows and amphipods. Other studies indicate that freshwater invertebrates, especially crustaceans, are adversely affected at lower Kelthane concentrations than pests such as mosquito larvae [*25*] and that sublethal effects of this pesticide to saltwater forms [*26*] occur at concentrations below those used in the present study. Concentrations of Pydrin (a mosquito larvicide) that caused effects in this test were lower than the LC_{50} values for several species of mosquito larvae which ranged from 0.9 to 7 μg/litre [*6,7*]. These and other studies [*8*] have shown that nontarget organisms such as mayflies and dragonflies are markedly affected or eliminated when Pydrin is applied for mosquito control. Important fish food organisms such as amphipods, mayflies, stoneflies, and dipteran larvae have been shown [*27*] to be adversely affected in 1 to 4 days at concentrations as much as 20 times lower than those which affected the larval fathead minnows in this test. The margin of safety (LC_{50}–90 fish/LC_{50}–90 mosquito larvae) for rainbow trout and other fish species [*5*] was indicated to be extremely low based on their high sensitivity to Pydrin.

Residue concentrations of Kelthane and Pydrin in fathead minnows and amphipods in this test increased with increased water concentrations after approximately 30 days. The BCFs obtained for fathead minnows and both pesticides were comparable. The BCF for Kelthane and amphipods was approximately three times higher than that calculated for fathead minnows after a similar exposure period. The higher ratio of surface area to mass of amphipods than of fish and the differences in lipids and metabolism of these organisms could account for the variation in Kelthane accumulation in these tests.

The BCFs calculated in this study for both fathead minnows and amphipods were directly related to the exposure concentrations in the water. Although no studies were found on the accumulation of Kelthane in aquatic organisms for comparisons to the present data, Anderson [*27*] showed that BCFs for Pydrin and snails decreased with increased exposure concentrations. This result with snails is normally observed in accumulation studies with a variety of species and compounds. No explanation can be given at this time for the direct relationship of BCFs to exposure concentrations in this study.

The BCF for Pydrin and fathead minnows was approximately six times higher than that found for snails exposed to this compound in Lake Superior water [*27*]. The BCFs for snails were calculated after their shells were removed, which would have resulted in lower values in these animals, assuming the shell absorbed the majority of this compound. This comparison differs from that for exposures of snails and fish with radiolabeled fenvalerate (common name for Pydrin) in a model ecosystem [*28*]. Although a direct comparison between these studies cannot be made, 30-day BCFs for this compound in the model ecosystem study were found to be the lowest in fish (carp) followed by daphnids, snails, and algae. The low BCF of fenvalerate in fish was attributed by these authors to the fish's ability to metabolize this compound and eliminate any metabolites more readily than the other food chain organisms. The fish were found to accumulate this compound in a short period of time, but they eliminated it when they were transferred to clean water for 25 days.

References

[*1*] Kapur, K., Kamaldeep, K., and Toor, H. S., *Bulletin of Environmental Contamination and Toxicology*, Vol. 20, 1978, pp. 438–442.

[*2*] Verma, S. R., Bansal, S. K., and Dalela, R. C., *Archives of Environmental Contamination and Toxicology*, Vol. 7, 1978, pp. 317–323.

[*3*] Sanders, H. O., "Toxicity of Pesticides to the Crustacean (*Gammarus lacustris*)," Technical Paper 25, U.S. Bureau of Sport Fisheries and Wildlife, Columbia National Fisheries Research Laboratory, Columbia, Mo., 1969.

[*4*] Sanders, H. O., "Toxicity of Some Insecticides to Four Species of Malacostracan Crustaceans," Technical Paper 66, U.S. Bureau of Sport Fisheries and Wildlife, Columbia National Research Laboratory, Columbia, Mo., 1972.

[5] Mulla, M. S., Navvab-Gojrati, H. A., and Darwazeh, H. A., *Environmental Entomology*, Vol. 7, 1978, pp. 428–430.
[6] Mulla, M. S., Navvab-Gojrati, H. A., and Darwazeh, H. A., *Mosquito News*, Vol. 38, No. 1, 1978, pp. 90–96.
[7] Mulla, M. S., Darwazeh, H. A., and Dhillon, M. S., *Mosquito News*, Vol. 40, No. 1, 1980, pp. 6–12.
[8] Miura, T., Takahashi, R. M., and Mulligan, F. S., III, *Proceedings*, the California Mosquito and Vector Control Association, Vol. 45, 1977, pp. 137–139.
[9] McKim, J. M., *Journal of the Fisheries Research Board of Canada*, Vol. 34, No. 8, 1977, pp. 1148–1154.
[10] Holcombe, G. W. and Phipps, G. L., "The Acute Toxicity of Kelthane, Dursban, Disulfoton, Pydrin, and Permethrin to Fathead Minnows (*Pimephales promelas*) and Rainbow Trout (*Salmo gairdneri*)," manuscript in preparation, U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn.
[11] *Standard Methods for the Examination of Water and Wastewater*, 14th ed., American Public Health Association, Washington, D.C., 1975.
[12] Benoit, D., Mattson, V. R., and Olson, D. L., "A Continuous Flow Mini-Diluter System for Toxicity Testing," U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn., manuscript in press, *Water Research*.
[13] Sprague, J. B., *Water Research*, Vol. 3, 1969, pp. 793–821.
[14] Phipps, G. L. and Holcombe, G. W., "Saturator System for Generating Toxic Water Solution for Aquatic Bioassays," U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn., manuscript in press, *Progressive Fish Culturist*.
[15] Veith, G. D. and Comstock, V. M., *Journal of the Fisheries Research Board of Canada*, Vol. 32, No. 10, 1975, pp. 1849–1851.
[16] *Pesticide Analytical Manual, Vol. I, Methods Which Detect Multiple Residues*, U.S. Department of Health, Education, and Welfare, U.S. Food and Drug Administration, Washington, D.C., 1972.
[17] Steel, R. G. D. and Torrie, J. H., *Principles and Procedures of Statistics with Special Reference to the Biological Sciences*, McGraw-Hill, New York, 1960.
[18] Jarvinen, A. W. and Tanner, D. K., "Toxicity of Selected Controlled Release and Corresponding Unformulated Technical Grade Pesticides to the Fathead Minnow (*Pimephales promelas*)," U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, Minn., manuscript in press, *Environmental Pollution Series A*.
[19] Benoit, D. A., Puglisi, F. A., and Olson, D. L. "Evaluation of a Method for Conducting Early Life Stage Toxicity Tests with Fathead Minnows (*Pimephales promelas*)," U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn., manuscript in press, *Environmental Pollution*.
[20] Holcombe, G. W., Phipps, G. L., and Fiandt, J. T., "Effects of Four Phenol Compounds [Phenol, 2,4-Dimethylphenol, 2,4-Dichlorophenol, and Pentachlorophenol] on Embryo, Larval, and Early-Juvenile Fathead Minnows (*Pimephales promelas*)," Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn., manuscript in press, *Archives of Environmental Contamination and Toxicology*.
[21] Wedemeyer, G., *Comparative Biochemistry and Physiology*, Vol. 26, 1968, pp. 271–279.
[22] Guiney, P. D., Lech, J. J., and Peterson, R. E., *Toxicology and Applied Pharmacology*, Vol. 53, 1980, pp. 521–529.
[23] Veith, G. D., DeFoe, D. L., and Bergstedt, B. V., *Journal of the Fisheries Research Board of Canada*, Vol. 36, 1979, pp. 1040–1048.
[24] *Pesticide Manual, Basic Information on the Chemicals Used As Active Components of Pesticides*, 2nd ed., H. Martin, Ed., British Corp. Protection Council, Worcester, England, 1971, p. 172.
[25] Rawash, I. A., Gaaboub, I. A., El-Bayar, F. M., and El-Shazli, A. Y., *Toxicology*, Vol. 4, 1975, pp. 133–144.
[26] Sharp, J. W., Sitts, R. M., and Knight, A. W., *Marine Biology*, Vol. 50, 1979, pp. 367–374.
[27] Anderson, R. L., "Effects of Fenvalerate and Permethrin on Non-Target Aquatic Invertebrates," manuscript in preparation, U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn.
[28] Ohkawa, H., Kikuchi, R., and Miyamoto, J., *Journal of Pesticide Science*, Vol. 5, 1980, pp. 11°22.